IV. Methods of Preparation

Methionine sulfone has been prepared by the oxidation of DL-methionine with hydrogen peroxide and by electrolysis of a solution of methionine or methionine sulfoxide.[14,15]

LINOLEIC ACID AND METHYL LINOLEATE

$$CH_3—(CH_2)_4—CH{=}CH—CH_2—CH{=}CH—(CH_2)_7—CO_2H$$

Mol. wt. 280.4 ($C_{18}H_{32}O_2$)

$$CH_3—(CH_2)_4—CH{=}CH—CH_2—CH{=}CH—(CH_2)_7—COOCH_3$$

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Checked by W. E. M. LANDS and H. E. CARTER, University of Illinois, Urbana.

I. Principle

$$\text{Safflowerseed oil} \xrightarrow[\text{(or methanolysis)}]{\text{Saponification}} \text{Fatty acids (or methyl esters)} + \underset{OH}{CH_2}—\underset{OH}{CH}—\underset{OH}{CH_2}$$

$$\text{Fatty acids (or methyl esters)} + H_2NCONH_2 \xrightarrow[\text{complex formation}]{\text{Selective}} \text{Complexed acids (or esters)} + \text{Free linoleic acid (or ester)}$$

ordinate scale the exact amount of NaOH equivalent to the sample titrated.

Analysis of DL-methionine sulfone:

		C	H	N	Equiv. Wt.
$C_5H_{11}O_4NS$	Calculated	33.14	6.12	7.72	181.2
	Found	33.18	6.19	7.73,7.65	181.3,179.3, 182.8

Analysis of DL-ethionine sulfone:

		C	H	N	Equiv. Wt.
	Calculated	36.9	6.71	7.17	195.2
	Found	36.8	6.88	7.08	194.1

[14] Saburo Miduno, *J. Chem. Soc., Japan*, **63**, 1317 (1942) [*C. A.*, **63**, 3057d (1942)].
[15] Yoshitaro Takayama and Saburo Miduno, *J. Chem. Soc., Japan*, **63**, 485 (1942) [*C. A.*, **41**, 3055f (1942)].

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Urea forms crystalline complexes preferentially with the saturated and monounsaturated components of a mixture of fatty acids (or methyl esters). If the quantity of urea used is sufficient to precipitate only these two types, the polyunsaturated components will concentrate in the non-complex (filtrate) fraction.

II. Starting Material

Safflowerseed oil[2] (iodine number, 148; composition: 78% linoleic; 12% oleic; 9.5% saturated; and 0.5% conjugated diene glycerides) was used in this preparation.[3]

III. Procedure

A. Linoleic acid. Safflowerseed oil fatty acids are prepared from the oil by the rapid saponification technique previously described under the isolation of oleic acid.[4] From 2.5 kg. of oil, 2300–2340 gm. of pale yellow fatty acids are obtained. The yield is 98–100%.

A solution of 880 gm. of urea[5] in 2.7 l. of methanol is prepared in a suitable container by heating on the steam bath. (A 2½-gal. Pyrex bottle is satisfactory.) To the solution, 1 kg. of safflowerseed oil fatty acids (previously warmed to 60°) is added and the mixture is heated and stirred only until complete solution is attained. The solution is then allowed to cool to room temperature (25°) overnight, and the crystalline material is separated by suction filtration. The filtrate is transferred to a large evaporating dish and evaporated to dryness in a hood while a brisk stream of nitrogen is passed over the surface of the mixture through an inverted funnel. Several volumes of warm water

[2] Refined and bleached safflowerseed oil was obtained from the Chemurgy Department of the University of Nebraska. It is now available commercially. The checkers obtained the oil from Welch, Holme, and Clark Co., 439 West St., New York 14, New York.
[3] Corn oil is also a satisfactory starting material, but yields are slightly lower.
[4] *Biochem. Preparations*, **2**, 100 (1952).
[5] Approximately 4 gm. of urea are employed per gram of acid to be precipitated.

containing about 30 ml. of 6 *N* hydrochloric acid[6] are added to dissolve the excess urea. The mixture is transferred to a separatory funnel, and the insoluble oil is dissolved in several volumes of petroleum naphtha, hexane fraction, boiling range 63–70°. The aqueous layer is discarded, and the upper layer is washed 3 times with water to remove residual urea. The upper layer is transferred to a distilling flask, and the solvent is recovered, the last traces being removed under reduced pressure at 100° while a stream of nitrogen is passed through the liquid. The linoleic acid concentrate is an amber oil which weighs 780–800 gm.

The linoleic acid concentrate is distilled under reduced pressure, using a Claisen head which contains a layer of Raschig rings[7] ½ in. deep to serve as a baffle to prevent splashing over. After separating a fore-run boiling at 155–168°/0.2 mm., which amounts to about 10% of the potcharge,[8] the main fraction, boiling at 165–168°/0.2 mm. or 195–199°/1 mm. (n_D^{30} 1.464–1.465 and n_D^{50} 1.456–1.458), is obtained as an almost colorless liquid, which weighs 600–625 gm. and has an iodine number of 173–177. Its composition is 92–95% linoleic acid and 5–8% oleic plus saturated acids.[9] The yield is 71–76% of the linoleic acid present in the safflowerseed oil used.

B. Methyl linoleate. Three grams of freshly cut metallic sodium are dissolved in l. of anhydrous methanol. One kilogram of safflowerseed oil is added, and the solution is refluxed for 30 minutes. Twelve hundred grams of urea and 5 l. of methanol are then added, and the mixture is boiled until a homogeneous solution[10] is obtained. The solution is cooled to 0–5° overnight, and the crystalline urea complexes are separated by suction filtration. The methyl esters in the non-complex (filtrate) fraction are isolated as described above for linoleic acid. The methyl linoleate concentrate is a yellow oil which weighs 720–750 gm.

Fractional distillation yields a small fore-run which weighs about 25 gm. boiling at 180–185°/4 mm., followed by the main fraction, boiling at 185–189°/3–4 mm. (n_D^{50} 1.448), obtained as a colorless oil which weighs 620–640 gm. and has an iodine number of 165–168. Its composition is 92–95% methyl linoleate and 5–8% of methyl esters of oleic and saturated acids. The yield is 80–85% of the linoleic acid present in the safflowerseed oil used.

[6] Small amounts of ammonium soaps are apparently formed, and, if they are not decomposed before solvent extraction, emulsions are usually obtained when the solvent is added.

[7] Other suitable materials can also be used.

[8] The formation of methyl esters during the urea complex precipitation procedure is unavoidable. These are lower-boiling than the free acids and concentrate in the fore-run.

[9] Small quantities of methyl esters may be present in the concentrate.

[10] The checkers found that an oily supernatant layer formed at this point but disappeared on standing in the cold room overnight.

IV. Properties and Purity of Product

The preparation by physical means of pure all *cis* linoleic acid and methyl linoleate from natural sources has been described by various workers.[11–14]

Linoleic acid: m.p. −5°; n_D^{50} 1.4588; iodine number 181.0.

Methyl linoleate: m.p. −35 to −34°; n_D^{25} 1.4593; iodine number 172.4.

V. Methods of Preparation

The classical method for preparing linoleic acid or methyl linoleate consists in bromination of linoleic acid-rich mixtures of fatty acids followed by debromination with zinc in methanol or other solvents.[15–17] Because the iodine number of the resulting products frequently agrees with the theoretical value, it was assumed for many years that they were pure and essentially

[11] J. Frankel and J. B. Brown, *J. Am. Chem. Soc.*, **63**, 1483 (1941); J. S. Frankel, W. Stoneburner, and J. B. Brown, *J. Am. Chem. Soc.*, **65**, 259 (1943).

[12] C. E. Swift, W. G. Rose, and G. S. Jamieson, *Oil & Soap*, **20**, 249 (1943).

[13] R. W. Riemenschneider, S. F. Herb, and P. L. Nichols, Jr., *J. Am. Oil Chemists' Soc.*, **26**, 371 (1949).

[14] T. P. Hilditch, C. B. Patel, and J. P. Riley, *Analyst*, **76**, 81 (1951).

[15] A. Rollett, *Z. physiol. Chem.*, **62**, 410 (1909).

[16] J. W. McCutcheon, *Org. Syntheses*, **22**, 75 (1942).

[17] R. T. O'Connor, D. C. Heinzelman, M. Caravella, and S. T. Bauer, *Oil & Soap*, **23**, 5 (1946).

identical with the natural product. With the availability first of ultraviolet and then of infrared spectrophotometric techniques, it became evident that *cis,cis*-octadecadienoic acid was not the sole component of the mixture and that geometrical and/or positional isomers were present in significant amounts (12%).[18] By multiple low-temperature fractional crystallization of the debromination products it was possible apparently to obtain a pure product having the natural configuration, but yields were low. Subsequently it was shown that the bromination-debromination steps could be eliminated and that pure all *cis* linoleic acid could be obtained from linoleic-rich oils by repeated low-temperature crystallization.[10] More recently chromatographic techniques have been successfully applied to the preparation of pure methyl linoleate,[11,12] but these techniques are still limited to relatively small-scale operations because of the low capacity of adsorption columns.

The present procedure is a simplification of a published method.[19] Although pure products are not obtained, the natural configuration is unaltered, large quantities of concentrate can be prepared quickly without the use of elaborate equipment or expensive chemicals, and yields are good. If higher purity is required, the chromatographic or crystallization methods can be employed on the concentrates. For many purposes, the 92–95% concentrates are satisfactory without additional purification.

Linolenic acid (or methyl esters) can be prepared from perilla oil by essentially the same procedure used for linoleic acid. The resulting product is somewhat less pure (85–87% linolenic acid, 5–6% linoleic acid, 2–5% oleic acid, 2–8% saturated acid).

[18] N. L. Matthews, W. R. Brode, and J. B. Brown, *J. Am. Chem. Soc.*, **63**, 1064 (1941).

[19] D. Swern and W. E. Parker; *J. Am. Oil Chemists' Soc.*, **30**, 5 (1953).

D-GLUTAMIC ACID

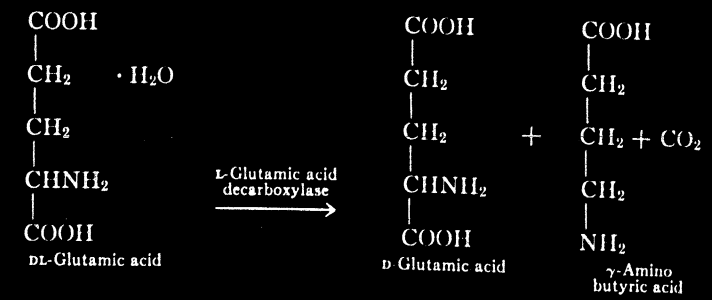
$HOOCCH(NH_2)CH_2CH_2COOH$

Mol. wt. 147.13 ($C_5H_9NO_4$)

Submitted by WILLIAM R. SHERMAN, Noyes Chemical Laboratory, University of Illinois, Urbana.

Checked by WILLIAM R. RUEGAMER, Veterans Administration Hospital and State University of New York, Medical College, Syracuse.

I. Principle



Certain microorganisms develop a powerful L-glutamic decarboxylase when properly grown.[1] Acetone drying of these cells makes the enzyme available for *in vitro* action on glutamic acid. When the enzyme is allowed to act on DL-glutamic acid, only the L-isomer is decarboxylated to γ-aminobutyric acid, leaving the D-glutamic acid unaffected. Since γ-aminobutyric acid is extremely soluble in water at all *p*H's, and since D-glutamic acid is only slightly soluble at its isoelectric point, separation is easily effected.

II. Starting Materials

L-Glutamic acid was racemized by a procedure derived from that of Dunn and Stoddard.[2] The modified procedure[3] is simple,

[1] W. W. Umbreit and I. C. Gunsalus, *J. Biol. Chem.*, **159**, 333 (1945).

[2] M. S. Dunn and M. P. Stoddard, *Biochem. Preps.*, **2**, 69 (1952).

[3] In a typical run, 1 kg. of General Mills 99% L-glutamic acid is heated for